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Preservation of *Listeria monocytogenes* and other pathogenic foodborne bacteria on silica gel

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Preservation of *Listeria monocytogenes* on silica gel was examined as a means of storing and transporting bacterial cultures. *Listeria monocytogenes* remained viable through 168 d of storage on silica gel at room and refrigeration temperatures. The viability of *Escherichia coli* O157 : H7, *Salmonella typhimurium* and *Staphylococcus aureus* after storage on silica gel was determined and compared with earlier reports.

The preservation of fungal spores or mycelium for several years on anhydrous silica gel was reported by Perkins (1962). Grivell and Jackson (1969) and Trollope (1975) successfully applied the methods of Perkins to the preservation of bacteria. We have found silica gel preservation to be a simple and reliable method of storing *Listeria monocytogenes* for transport to other laboratories. However, we had no data on the length of time the organism remained viable, and previous studies on bacterial preservation on silica gel did not include *L. monocytogenes*. Accordingly, the objective of the current study was to assess the viability of *L. monocytogenes* preserved on silica gel over the course of approximately half a year. Additionally, the viability of three other foodborne pathogens, *Staphylococcus aureus*, *Escherichia coli* O157 : H7 and *Salmonella typhimurium*, was evaluated and compared with earlier reports.

Materials and Methods

MICRO-ORGANISMS

Escherichia coli O157 : H7/strain #933, *Listeria monocytogenes* Scott A and *Staphylococcus aureus* CAMP were maintained as stock cultures in Brain Heart Infusion Broth (BHI) (Difco) at 4°C and subcultured monthly. *Salmonella typhimurium* 14028 was kept in Tryptic Soy Broth (TSB) (Difco) at 4°C and subcultured monthly.

PREPARATION OF SILICA GEL

Silica gel (28–200 mesh, pore diameter = 2·2 nm, Sigma Chemical Co.) was dispensed into 140 6 × 1·5 cm screw cap glass vials so that each vial was half full (approximately 3·25 g per vial). Vials were capped and baked at 100°C for 3 d.

CULTURE CONDITIONS

A loopful of each stock culture was transferred to one of four 250-ml Erlenmeyer flasks containing 50 ml sterile BHI broth and incubated 18–24 h on a rotary shaker (150 rev min^{-1}) at 37°C. The cultures were transferred to 250-ml sterile plastic centrifuge bottles and centrifuged at 10 240 × g for 20 min at 4°C. The supernatants were discarded. A loopful of the pellet from each bacterial strain was transferred to each of 30 silica gel vials. The initial level of bacteria was approximately $3{\cdot}08 \times 10^6$ cfu g^{-1}. After shaking to evenly distribute the cells

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within the vial, 15 vials per strain were stored at 5°C and room temperature (22–24°C). Uninoculated vials of silica gel stored at room temperature served as negative controls.

TESTING VIABILITY

Periodically, the contents of one of the silica gel vials were transferred to 50-ml sterile BHI broth and incubated 18–24 h on a rotary shaker (150 rev min^{-1}) at 37°C. Growth was noted, and a loopful from each flask was streaked onto Brain Heart Infusion Agar (BHIA) (Difco). Plates were incubated for 18–24 h at 37°C and examined for growth as a pure culture.

Results and Discussion

Survival of four organisms, *L. monocytogenes*, *Salm. typhimurium*, *E. coli* and *Staph. aureus*, was examined over 168 d of storage on silica gel at room and refrigeration temperatures. *Staphylococcus aureus* and *L. monocytogenes* remained viable throughout the study at both room and refrigeration temperatures (Table 1). *Salmonella typhimurium* survived 168 d under refrigeration. All samples stored at room temperature were positive except on day 98. The lack of detectable *Salm. typhimurium* from the 98 d room temperature sample may reflect inadequate inoculation of that particular vial. *Escherichia coli* was viable during the first 7 d of storage at room temperature. After 7 d, recovery was erratic and by 28 d of storage, no viable cells were recovered. Refrigerated *E. coli* remained viable through 70 d of storage, but recovery was erratic after 84 d of storage.

These results demonstrate the successful preservation of *L. monocytogenes* on silica gel for at least 24 weeks at room and refrigeration temperatures. The survival periods of the foodborne pathogens, *Staph. aureus* and *Salm. typhimurium*, agree with the survival periods for those organisms previously reported in the literature. However, *E. coli* O157 : H7 did not survive as long on silica gel at 5°C as the non-pathogenic *E. coli* strains used in earlier studies. Trollope (1975) reported a survival period of 315 d at 4°C for *E. coli* NCIB 8277, and *E. coli* K12 was reported viable up to 462 d at 4°C (Grivell and Jackson 1969). The variations in survival periods may reflect strain differences as well as differences in cultural conditions, suspending media, inoculum size, and inoculum age. The short survival period of 7 d for *E. coli* O157 : H7 at room temperature agrees with the survival period reported for *E. coli* NCIB 8277 (Trollope 1975).

The current results support earlier observations that Gram-positive bacteria tend to survive storage on silica gel better than most Gram-negative bacteria (Trollope 1975). This tendency is exemplified by the successful preservation of lactic acid bacteria and their acidifying activity on anhydrous silica gel for 3 years (De Silva *et al.* 1983). Silica gel has also been used

Table 1. Recovery of viable cells from cultures stored on silica gel

	Salmonella typhimurium		*Escherichia coli*		*Staphylococcus aureus*		*Listeria monocytogenes*		
Day	RT	5°C	RT	5°C	RT	5°C	RT	5°C	Negative control
0	+	+	+	+	+	+	+	+	−
7	+	+	+	+	+	+	+	+	−
14	+	+	−	+	+	+	+	+	−
21	+	+	+	+	+	+	+	+	−
28	+	+	−	+	+	+	+	+	−
42	+	+	−	+	+	+	+	+	−
56	+	+	−	+	+	+	+	+	−
70	+	+	−	+	+	+	+	+	−
84	+	+	−	−	+	+	+	+	−
98	−	+	−	+	+	+	+	+	−
112	+	+	−	−	+	+	+	+	−
126	+	+	−	−	+	+	+	+	−
140	+	+	−	−	+	+	+	+	−
154	+	+	−	+	+	+	+	+	−
168	+	+	−	−	+	+	+	+	−

RT, Room temperature (22–24°C).

successfully to preserve plant-pathogenic bacteria (Leben 1982).

Mention of brand or firm names does not constitute an endorsement by the US Department of Agriculture over others of a similar nature not mentioned.

References

De Silva, M.C.T., Tessi, M.A. and Moguilevsky, M.A. 1983 Preservation of lactic acid bacteria on anhydrous silica gel for three years. *Journal of Food Protection* **46 (8)**, 699–701.

Leben, C. 1982 Preservation of plant-pathogenic bacteria on silica gel. *Plant Disease* **66**, 327.

Grivell, A.R. and Jackson, J.F. 1969 Microbial culture preservation with silica gel. *Journal of General Microbiology* **58**, 423–425.

Perkins, D.D. 1962 Preservation of neurospora stock cultures with anhydrous silica gel. *Canadian Journal of Microbiology* **8**, 591–594.

Trollope, D.R. 1975 The preservation of bacteria and fungi on anhydrous silica gel: an assessment of survival over four years. *Journal of Applied Bacteriology* **38**, 115–120.

Formation of histamine and tyramine by some lactic acid bacteria in MRS-broth and modified decarboxylation agar

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Production of histamine and tyramine was studied in lactic acid bacteria (LAB) used for starter cultures (13 strains), isolated from commercial starter preparations (four strains) and isolated from dry sausages (10 strains). The only amine-positive starter strain detected either with modified decarboxylation medium or with an HPLC method from MRS-broth was *Lactobacillus brevis*, traditionally used for bakery products. According to these *in vitro* results, the most common meat starter LABs in Finland can be regarded as safe with respect to histamine and tyramine production.

Biogenic amines are basic nitrogenous compounds formed mainly by decarboxylation of amino acids or by amination and transamination of aldehydes and ketones. Especially histamine (often associated with scombroid poisoning) and tyramine (inducing e.g. migraine) may represent a food poisoning hazard increased by potentiating factors such as amine oxidase inhibiting drugs, alcohol, other food amines and gastrointestinal diseases (e.g. Askar and Treptow 1986).

Biogenic amines are often found in fermented foods such as dry sausages and cheeses, in which lactic acid bacteria (LAB), non-starter (NSLAB) as well as starter LAB, ferment the product. Amine-producing LABs such as *Lactobacillus brevis*, *Lact. buchneri*, *Lact. divergens*, *Lact. hilgardii*, *Lact. carnis* and *Lact. curvatus* have been isolated from meat and meat products (Edwards *et al.* 1987; Tschabrun *et al.* 1990). However, many commercial starter LABs are regularly used without any knowledge of their amine production potential.

Moeller (1954) used a pH-based differential medium to examine the distribution of amino acid decarboxylase activity in Enterobacteriaceae. Niven *et al.* (1981) modified Moeller's decarboxylase medium and developed a differential agar medium for qualitative detection of histamine-producing bacteria. This medium, based on the colour change of bromcresol purple, has been successfully used in several studies for the detection of amine-producing enterobacteria in fish and fish products (e.g. Chen *et al.* 1989). However, because of its composition it has only limited value for the study of LABs. Joosten and Northolt (1989) modified Niven medium to enhance the growth of LABs of cheeses. In our previous studies most of the starter cultures grew well on this medium, but in some cases great difficulties were experienced in inducing NSLABs to grow and/or produce a positive reaction (unpublished data).

The purpose of this investigation was to study the amine production of the most common starter LABs used for meat products in Finland as well as to develop an agar medium for the detection of production of tyramine and histamine by LABs.

Materials and Methods

Thirteen pure cultures of LAB strains used for starter cultures were kindly provided by Rudolf Müller & Co., Germany and Christian Hansen's Laboratory, Denmark. Four additional LAB